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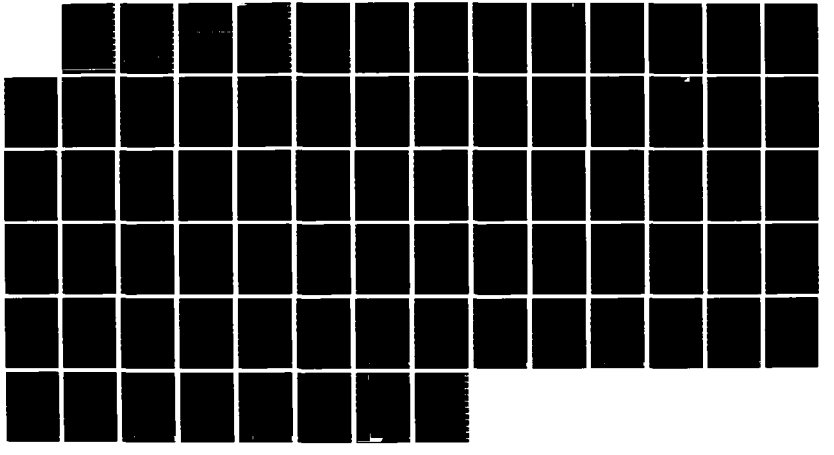

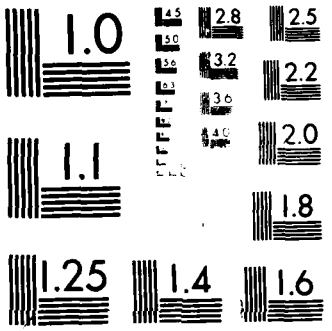

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USA DEPARTMENT OF THE NAVY, OFFICE OF NAVAL RESEARCH (ONR)

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US-ISRAEL BINATIONAL SCIENCE FOUNDATION (BSF)

Joint Workshop

on

ASPECTS OF HALOPHILISM

PROGRAM / ABSTRACTS

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Joint Workshop

on

ASPECTS OF HALOPHILISM

Organizing Committee:

Henryk Eisenberg — *Weizmann Institute of Science, Rehovot*

Moshe Mevarech — *Tel-Aviv University, Tel-Aviv*

Aharon Oren — *The Hebrew University of Jerusalem, Jerusalem*

Leo Parks — *North Carolina State University, Raleigh*

Venue: The Israel Academy of Sciences and Humanities,
Albert Einstein Square, Talbieh, Jerusalem

JERUSALEM, MARCH 23-28, 1986

C O N T E N T S

SUNDAY, MARCH 23, 1986

09:00-09:30 Opening Session

Introduction : HENRYK EISENBERG, Weizmann Institute
Opening Address : FRED SAALFELD, Director of Research Programs, ONR
Welcoming Address: ZEEV ROTEM, Director, BSF

A. Ecology and Taxonomy

Chairman: D.J. KUSHNER (Ottawa)

09:30-10:10 A. OREN (Jerusalem).
Fifty years of microbiological studies in the Dead Sea: 1936-1986.

10:10-10:50 W.D. GRANT (Leicester)
The ecology and taxonomy of the archaebacterial halophiles;

10:50-11:30 L. I. HOCHSTEIN, R. L. Mancinelli and G. A. Tomlinson (Moffett Field, CA)
On the occurrence of denitrification in the extreme halophilic bacteria;

11:30 Coffee

12:00-12:40 B. JAVOR (San Diego, CA)
CO_2 fixation in halobacteria;

12:40-13:20 A. H. Latorella and R. D. SIMON (Geneseo, N.Y.)
Comparison of Dunaliella strains: Approaches to a modern taxonomy;

13:30 Lunch

B. Structural Aspects

Chairman: W. STOECKENIUS (San Francisco)

15:00-15:40 A.T. MATHESON (Victoria)
The evolution of the ribosome from moderate and extreme halophiles;

15:40-16:20 A. YONATH, M. Saper, M. Shoham, A. Shevack, I. Makowski, T. Arad and H. Wittmann (Rehovot, Berlin)
Structural studies on crystalline ribosomal particles from Halobacterium marismortui.

16:20 Coffee

16:50-17:30 M. SHOHAM, M. Kimura, R. Reinhardt and J. Dijk (Rehovot, Berlin)
Purification and characterization of ribosomal proteins from the 30S subunit of the extreme halophile Halobacterium marismortui.

MONDAY, MARCH 24

13:00 Lunch

B. Structural Aspects (Continuation)

Chairman: H.G. WITTMAN (Berlin)

14:30-15:10 M. Shoham, J. H. Brown and J. L. SUSSMAN (Rehovot)
X-ray structural studies on a 2Fe-2S ferredoxin from halobacteria of the Dead Sea;

15:10-15:50 G. Zaccai, E. Wachtel and H. EISENBERG (Grenoble, Rehovot)
Solution structure of halophilic malate dehydrogenase from small angle neutron and X-ray scattering and ultracentrifugation measurements;

15:50 Coffee

16:20-17:00 F. WIELAND (Stanford/Regensburg)
Halobacterial glycoproteins;

17:00-17:40 S. Cohen, M. Shilo and M. KESSEL (Jerusalem)
The effect of lowered Ca^{++} and NaCl concentration on the cell wall of *Halobacterium volcanii* from the Dead Sea;

17:40-18:20 B. Z. Ginzburg and H. MORGAN (Jerusalem/Oxford)
Dielectric properties of some halophilic bacteria;

1

TUESDAY, MARCH 25, 1986

C. Energetics.

Chairman: M. SHILO (Jerusalem)

09:00-09:40 W. STOECKENIUS (San Francisco, CA)
Structure and function of the retinal pigments in halobacteria;

09:40-10:20 J. K. LANYI (Irvine, CA)
The mechanism of active chloride accumulation in Halobacterium halobium.

10:20-11:00 A.M. Kleinfeld, J.D. LeGrange and S.R. CAPLAN (Boston, Rehovot)
Tryptophan imaging of bacterio-opsin reconstituted into lipid vesicles.

11:00 Coffee

Chairman: R. D. SIMON (Geneseo, N.Y.)

11:30-12:10 M. ENGELHARD, K.-D. Kohl and B. Hess (Dortmund)
The metal binding site of bacteriorhodopsin (bR);

12:10-12:50 D.J. KUSHNER (Ottawa)
Studies of active transport as a means of understanding salt requirements and salt tolerance;

12:50-13:30 M. Bental, A. Ben-Amotz, M. Avron and H. DEGANI (Rehovot)
^{31}P NMR studies of Dunaliella.

13:40 Lunch

D. Taxis

Chairman: M. OTTOLENGHI (Jerusalem)

15:30-16:30 D. OESTERHELT, M. Alam and W. Marwan (München)
Halobacterial motion and taxis.

16:30-17:10 E.K. WOLFF, R.A. Bogomolni, B. Hess and W. Stoeckenius (Dortmund, San Francisco)
Phototaxis in Halobacterium halobium.

17:10 Coffee

THURSDAY, MARCH 27, 1986

E. Mechanisms of Halotolerance

Chairman: J. K. LANYI (Irvine, CA)

09:00-09:40 R. H. VREELAND (New Orleans)
NaCl stimulated physiological changes in salt tolerant bacteria.

09:40-10:20 S. Ken Dror and Y. AVI-DOR (Haifa)
The role of monovalent cations in the regulation of respiration of a halotolerant microorganism.

10:20 Coffee

10:50-11:30 M. KOGUT and N.J. Russell (London, Cardiff).
Phenotypic adaptation of a moderate halophile to altered salinity and osmotic stress - the role of phospholipids.

11:30-12:10 A. BEN AMOTZ (Rehovot)
Mechanism of halotolerance in Dunaliella: Determination of glycerol and inorganic ion content.

12:10-12:50 M. Ginzburg and B. Z. GINZBURG (Jerusalem/Oxford)
Evidence for the metabolic control of Na^+ and Cl^- in Dunaliella grown at constant salinity.

13:00 Lunch

F. Molecular Genetics

Chairman: L. PARKS (Raleigh, N.C.)

14:10-14:50 K. Ebert, W. Goebel C. Hanke, A. Moritz, U. RDEST and B. Surek (Würzburg)
Genome and gene structure in halobacteria.

14:50-15:30 C.J. DANIELS (Columbus, OH)
Transfer RNA gene structure and transcript processing in the archaebacterium Halobacterium volcanii.

15:30 Coffee

16:00-16:40 M. MEVARECH, I. Rozenshein, R. Werczberger, T. Zusman (Tel Aviv/Edmonton)
Genetic transfer in Halobacterium volcanii.

16:40-17:20 R. GUPTA (Carbondale, Illinois)
Transfer RNAs of Halobacterium volcanii.

17:20-18:00 F. PFEIFER (München)
Organization of the Halobacterium halobium genome.

18:00-18:20 R. SANCHEZ-LOPEZ, I. Hoffman-Kuhn and B. Jarry (Strasbourg)
Molecular characterization of a multi-copy plasmid isolated from a halophilic bacteria LPR3 SP.

FRIDAY, MARCH 28, 1986

F. Molecular Genetics (continuation)

Chairman: M. WERBER (Kfar-Saba)

09:00-09:40 M. BETLACH, D. Leong and H. Boyer (San Francisco, CA)
Genetic regulation of bacterio-opsin synthesis in Halobacterium halobium.

09:40-10:20 F. GROPP and W. Zillig (München)
Gene expression of Halobacterium halobium phage ϕH.

10:20-11:00 P. P. DENNIS (Vancouver)
Molecular characterization of genes encoding elements of the Halobacterium protein synthesis apparatus.

11:00 Coffee

Chairman: D. OESTERHELT (München)

11:30-13:30 Open Discussion

13:30-13:45 Closing remarks

13:45 Lunch.

5:00pm ous

FIFTY YEARS OF MICROBIOLOGICAL STUDIES IN THE DEAD SEA: 1936-1986

Aharon Oren

The Division of Microbial and Molecular Ecology
The Institute of Life Sciences
The Hebrew University of Jerusalem
Jerusalem 91904, Israel

Exactly fifty years have passed since Elazari-Volcani (Wilkansky) published his first account of the discovery of halophilic microorganisms in the Dead Sea [1]. Volcani's studies [2] have shown that the Dead Sea is far from being sterile as was thought before, but is inhabited by a variety of microorganisms: different types of bacteria, unicellular green algae and even protozoa [3].

Quantitative data on the biota of the Dead Sea before 1980 are scarce; a study by Kaplan and Friedman in the years 1963-1964 [4] showed high and rather constant community densities of red halobacteria (up to 9×10^6 cells/ml) in the upper water layers, while the green alga Dunaliella reached numbers of up to 4×10^4 cells/ml.

The planning of a water carrier connecting the Dead Sea with the Mediterranean renewed the interest in the biology of the Dead Sea, and we started a systematic survey of the lake's biota in the beginning of 1980.

Community densities of bacteria and algae were very small in the beginning of 1980, and increased sharply during the summer and autumn of that year to values of up to 2×10^7 cells/ml and 8.8×10^3 cells/ml, respectively [5,6]. This bloom remained restricted to the upper 10-15 m of the water column, separated from the deeper layers by a pycnocline and/or thermocline. This stratification was caused by the influx of large amounts of rain floods in the winter of 1979-1980, diluting the upper water layers by up to 10%. The bacterial bloom consisted for 80% of pleomorphic halobacteria resembling Halobacterium volcanii, and for 20% of rod-shaped halobacteria (H. sodomense type). Large concentrations of the purple pigment bacteriorhodopsin were found in the halobacterial community.

From August 1980 onwards the Dunaliella community started to decline till no more cells were observed in February 1982. Bacterial numbers remained high in the upper water layers (around $3-5 \times 10^6$ cells/ml) till November-December 1982, when an overturn of the water column caused the mixing of the water layers [7]. No new blooms of halobacteria and of Dunaliella were observed in subsequent years (1983-1985).

Simulation experiments in the laboratory and under field conditions [8] demonstrated that the factors limiting development of Dunaliella in the Dead Sea are the too high salinity of the water (or rather the too high concentrations of divalent cations), and lack of available phosphate. Bacterial development is possible when suitable organic substrates (in nature supplied by photosynthesis of Dunaliella) and phosphate are present, and is also stimulated by increasing dilution of the Dead Sea water. These results explain the

occurrence of the summer bloom in 1980, and the lack of similar blooms in the following four seasons, and enable us to predict the development of future blooms from the physical structure and the chemical properties of the lake's water column.

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The Ecology and Taxonomy of the Archaebacterial Halophiles

W. D. Grant

Department of Microbiology, University of Leicester,

Leicester LE1 7RH, U. K.

In equatorial regions, many naturally - occurring highly saline brines have high populations of characteristic archaebacteria containing C_{50} carotenoids that impart a red colour to these brines. There are many different kinds of natural brines, compositions being influenced by the geology and climate of particular areas, but at least three distinct types are dominated by these archaebacterial halophiles (of different types), particularly as salt concentrations approach saturation. Salt lakes are usually highly eutrophic due to primary productivity by eukaryotic algae in some cases, or anoxygenic phototrophic bacteria in othes cases. Archaebacterial halophiles grow primarily as aerobic organotrophs on the products of primary production but they have other strategies under adverse conditions that will be discussed.

Many archaebacterial halophiles are biochemically inert, although a few sugar - utilizing strains are known (1,2) and in general standard biochemical tests have not proved very useful in characterizing isolates, even when quite extensive numerical

taxonomic studies have been carried out. Traditionally, only two major groups have been recognized - the coccoid isolates (halococci) and the rod - shaped or pleomorphic isolates (halobacteria). Recently, the discovery of haloalkaliphilic archaebacteria in soda lakes (3,4) has pointed to considerable diversity within the general halophile phenotype and prompted a reappraisal of the entire group. The rod - shaped haloalkaliphiles (natronobacteria) and coccoid haloalkaliphiles (natronococci) are clearly distinct from each other and from 'classical' halobacteria and halococci as determinied by lipid analyses and nucleic acid hybridization studies (5,6). In particular, the haloalkaliphiles were the first archaebacteria shown to have sesterterpanyl (C_{25}) isopranoid chains in ether linkage to glycerol (7) although these structures are now known to be present in other halophiles albeit in smaller amount (5). Chemotaxonomic analyses of these kinds further point to 6 or 7 major taxa (probably at genus level) within the 'classical' halobacteria, but indicate that isolates of halococci are very similar to one another (8). Recently sequence analyses of ribosomal RNA genes have been consistent with the view that the archaebacterial halophiles are a diverse group. The implications for the taxonomy of the group will be discussed.

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(1) Tomlinson, G. A. & Hochstein, L. I. (1976). Canadian Journal of Microbiology 22, 587-591.

(2) Gonzalez, C., Gutierrez, C. & Ramirez, C. (1978). Canadian Journal of Microbiology 24, 710-715.

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ON THE OCCURRENCE OF DENITRIFICATION IN EXTREMELY HALOPHILIC BACTERIA

Lawrence I Hochstein[a], Rocco L Mancinelli[a], and Geraldine A Tomlinson[b]

Planetary Biology Division, Ames Research Center, Moffett Field, CA 94035 [a] and Department of Biology, Santa Clara University, Santa Clara, CA 95053 [b]

Denitrification takes place under conditions of low oxygen tension resulting in the conversion of nitrate and nitrite to gaseous products, principally dinitrogen (1). Although the process is distributed among a variety of bacterial genera (2), evidence for its occurence in the *Halobacteriaceae*, the predominant microorganisms found in hypersaline enviroments (3), is equivocal. This seems incongruous in view of the low oxygen concentration characteristic of such enviroments.

During studies of the halobacterial flora present in local salterns, we suggested that the extreme halophiles, particularly those which use carbohydrates, could be categorized into 3 groups based on how nitrate was reduced (4). One of the proposed groups rapidly produced gas from nitrate, indicating that such organisms might be denitrifiers. Javor (5) reported that most of the extreme halophiles she isolated from widely separated salterns grew by anaerobic fermentation, although the possibility that some grew at the expense of nitrate reduction was not ruled out.

In order to determine if there indeed were extreme halophiles that could couple anaerobic growth to denitrification, 2 approaches were employed 1) we tested several representative extreme halophiles that produced gas when grown in the presence of nitrate for their ability to couple anaerobic growth to denitrification; 2) we attempted to isolate denitrifying halobacteria by anaerobic enrichment in the presence of nitrate. For the first part, three organisms were tested: the Ginzburg strain of *H. marismortui*, *H. vallismortis*, and *H. mediterraneii*. The following observations were made: none grew anaerobically in the absence of nitrate and in those cases where "anaerobic growth" was observed in the absence of nitrate, gas chromatographic analysis revealed the presence of oxygen; *H. marismortui* produced nitrite, nitrous oxide and dinitrogen while growing, but resting cells produced only nitrite and nitrous oxide; *H. vallismortis* produced considerable quantities of nitrite as well as nitrous oxide and dinitrogen, although the rates of gas production differed from those observed in the case of *H. marismortui*. Finally, in the case of *H. mediterraneii* dinitrogen was the principle product with only trace quantities of nitrite accumulating in the medium.

Denitrifying extremely halophilic bacteria were isolated from various salterns following anaerobic enrichment in nitrate-containing complex medium. The organisms grew anaerobically in the presence of nitrate and produced copious quantities of gas (6, Tomlinson and Hochstein, mss. in prep,). None of the organisms grew fermentatively; all were red chromogens; they grew over a wide range of salt concentrations (1.0 -4.5 M NaCl); and all utilized carbohydrates with the production of acid. Their growth was inhibited by aphidicolin, an inhibitor of halobacterial, but not eubacterial growth (7). The pattern of denitrification depended on the nitrate concentration. Dinitrogen was detected when growth took place in the presence of low nitrate concentrations (0.1%). In the presence of high nitrate concentrations (>0.25%), nitrite, nitrous oxide, as well as dinitrogen were detected. All of the organisms grew on nitrite although some better than others. In addition some of the strains that grew on nitrite produced nitric oxide.

The membrane-bound nitrate reductase from one of these organisms, *Halobacterium denitrificans* (8), reduced nitrate to nitrite when incubated in the presence of methyl viologen and dithionite. The enzyme could be stored for at least 2 days in the absence of added NaCl without any loss of activity. Beyond that time the experiment was terminated by bacterial contamination. The enzyme was also most active in the absence of added salt (NaCl = 60 mM). These salt-dependent properties were similar to those described by Marquez and Brodie (9) for a partially purified nitrate reductase obtained from an unidentified extreme halophile isolated from the Great Salt Lake. The nitrite reductase activity from *H. denitrificans* was distributed between the cytoplasmic and the membrane fractions. The assay conditions precluded defining the effect of salt concentration on enzyme activity since the assay was carried out in the presence of about 1 M salt (720 mM K^+ and 300 mM Na^+). The product of nitrite reductase activity and the apparent distribution of this enzyme depended on the reducing system that was used. In the presence of phenazine methosulfate and ascorbate, the membrane and cytoplasmic fractions produced nitric oxide. When dithionite (or methyl viologen and dithionite) were employed, only the membrane fraction was active, and the only product detected was nitrous oxide.

These results confirm: that several previously described organisms that produced gas when grown in the presence of nitrate were indeed denitrifiers; that denitrifying extreme halophiles are present in hypersaline enviroments and easily isolated; that the denitrifying enzymes from the halobacteria possess certain unexpected salt-dependent properties which make them interesting objects of study

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CO_2 fixation in halobacteria

Barbara Javor
Scripps Institution of Oceanography, A-002
University of California, San Diego
La Jolla, California 92093

Seven strains of extremely halophilic bacteria fixed CO_2 under light and dark conditions. Light enhanced CO_2 fixation in some strains but inhibited it in others. $^{14}CO_2$ assimilation in semi-starved cells was stimulated by NH_4^+ in six of the strains. Thiosulfate had no effect and sulfide and hydroxylamine were inhibitory. No RuBP carboxylase activity was detected. In most strains, pyruvate stimulated and succinate and acetate inhibited CO_2 fixation in semi-starved cells. The main ^{14}C-labeled α-keto acid detected after a 2-min incubation with $^{14}CO_2$ and pyruvate was pyruvate, suggesting that the carboxylation was primarily an exchange reaction with pyruvate. Propionate stimulated $^{14}CO_2$ incorporation in some strains, but inhibited it in others. Propionate-stimulated CO_2 fixation was sensitive to trimethoprim, an inhibitor of dihydrofolate reductase. Little or no α-ketobutyrate was detected among the early products of propionate-stimulated CO_2 fixation, indicating that this is probably not a reductive carboxylation (Danon and Caplan, 1977; Oren and Shilo, 1983). Glycine was the major amino acid synthesized during a 2-min incubation with NH_4^+, propionate, and $^{14}CO_2$. Alanine, glutamate, and aspartate were the major amino acids labeled during incubation with NH_4^+, HCO_3^-, and $^{14}CH_3CH_2COOH$. Due to problems of separating derivatized amino acids in a 4.28 M NaCl solution with radiolabeled propionate, it is uncertain whether glycine was also labeled during the assays.

A novel pathway for CO_2 fixation involving a glycine synthase reaction with CO_2, NH_4^+, and a methyl carbon derived from the β-carbon cleavage of propionate is tentatively proposed. This pathway resembles the reverse process of glycine fermentation described in Peptococcus glycinophilus (Barker et al., 1948; Robinson et al., 1973).

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Comparison of Dunaliella strains: Approaches to a Modern Taxonomy.

A. Henry Latorella and Robert D. Simon.
Department of Biology, SUNY-Geneseo, Geneseo, NY 14454, U.S.A.

Because of their ability to accumulate large amounts of potentially valuable organic compounds, and their ability to grow in saline waters which are normally of marginal value for agriculture, species of the euryhaline green alga Dunaliella may be useful for the bioconversion of solar energy. Indeed, "open-pond" culture of this organism is already taking place on three continents.

There is little modern information available on the taxonomy of this group, especially on the physiological and genetic variants available. This is unfortunate because there may be strains better suited for commercial production than those currently used. Valuable strains would be those that produce enhanced amounts of chemicals or that can be genetically manipulated. The latter is particularly important because successful production of biological products most often involves a program of strain improvement. In addition, if advances in biotechnology are to be applied to these eucaryotic algae, there will be a need to find Dunaliella strains amenable to the techniques of genetic engineering.

A program of Dunaliella strain comparison aimed at developing a physiological and genetic taxonomy of the group has been started. Strains were obtained from several collections as well as directly from groups working with the organism. Attempts have been made to piece together the strain histories although, because the current method for storing Dunaliella is as a growing culture, even strains of identical origin may have diverged when cultivated for long periods of time in different laboratories. The following characteristics have been examined:

1. The physiological properties of strains have been studied, including: salt tolerance ranges; nitrogen requirements; and sensitivity to drugs and detergents.

2. The electropherograms of total soluble proteins have been examined, and a program screening isozyme patterns in all strains is currently underway. This work will allow an analysis of the divergence of the strains identified as Dunaliella using the techniques of population biology.

3. An analysis of the pigments produced by strains and the amounts of other cellular isoprenoids is being carried out. The isoprene-based carotenoids represent one of the major salable products of Dunaliella, and perhaps there are strains with enhanced production rates or which make other similar compounds of commercial interest.

4. The strains are being examined to determine whether they might be useful in developing a genetic system, either using traditional algal genetics or with the techniques of plant biotechnology.

The Evolution of the Ribosome from Moderate and Extreme Halophiles

A.T. Matheson
Department of Biochemistry and Microbiology, University of Victoria
Victoria, British Columbia V8W 2Y2, Canada

We have been studying the structure of the ribosomes from the moderate halophile NRCC 71227 (a eubacterium) and from the extreme halophile Halobacterium cutirubrum (an archaebacterium). We have also been investigating the ribosome structure of a thermophilic methanogen, Methanobacterium thermoautotrophicum. This bacterium is an archaebacterium that does not require salt for growth but has an internal K^+ concentration close to 1 M (1). The ribosomal proteins from this bacterium show properties similar to those from the moderate halophiles (2).

Although the ribosomes from moderate and extreme halophiles are of similar size (70S) and contain the same number of ribosomal proteins, the shape of the afchaebacterial ribosomes are different from that of the eubacterial ribosomes (3). In addition, the r-proteins from the extreme halophiles are much more acidic, due mainly to an increased aspartate content in their proteins.

We have been studying the comparative structure of two ribosomal domains in the halophilic ribosome - the 5S rRNA-protein domain and the ribosomal 'A' protein domain. The most extensively studied protein has been the ribosomal 'A' protein, equivalent to L7/L12 in Escherichia coli. The 'A' protein from the moderate halophile NRCC 71227 shows a similar primary structure to other eubacterial 'A' proteins and is very different in structure to the 'A' protein from the extreme halophile H. cutirubrum. This protein is similar in structure to the 'A' protein from other members of the archaebacteria (Mb. thermoautotrophicum and Sulfolobus acidocaldar-

ius). The archaebacterial 'A' proteins show many features in common with the equivalent proteins in eukaryotes. Although a re-arrangement of the protein is required to line up the structural similarities between the archaebacterial and eubacterial 'A' proteins, the 'A' protein from the extreme halophile shows a significantly greater structural similarity to the eubacterial 'A' protein than do the 'A' proteins from the other archaebacteria.

If the archaebacteria are earlier than the eubacteria in the evolutionary scale it is possible that the 'A' protein from the extreme halophile gave rise to the 'A' proteins in the eubacteria (moderate halophiles). The data also suggests that the ancestral cell for the extreme halophiles may also have given rise to some of the methanogens. Even though these methanogens do not now require salt for growth the high level of internal salt and the physical properties of the ribosomal 'A' protein suggest these properties may be remnants of the more halophilic ancestral cell .

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Structural Studies on Crystalline Ribosomal Particles from Halobacterium marismortui.

A.Yonath, M.A.Saper, M.Shoham, A.Shevack*, I.Makowski*, T.Arad and H.G.Wittmann*

Dept. of Structural Chem., Weizmann Inst., Rehovot, and *MPI für Molekulare Genetik, W. Berlin

Ribosomes are distinct assemblies of proteins and RNA chains on which protein biosynthesis occurs in all organisms. The biochemical and physical properties of ribosomes are well characterized; however, full functional understanding requires a detailed molecular model. With this goal in mind we have grown, in vitro, several three-dimensional crystal forms, as well as two-dimensional sheets of intact ribosomal particles from eu- and halobacteria. Only active particles crystallize. In all cases, particles from dissolved crystals are active and contain all of their constituent proteins. In contrast to the short lifetime of isolated ribosomes, the dissolved crystalline particles sediment as intact particles even after several months of storage. The preservation of ribosome activity in the crystalline state agrees with the observation that ribosomes, in organisms exposed to stressful conditions, organize into periodic arrays for prolonged storage.

The best crystals are of the large (50S) ribosomal subunit. These particles consist of more than 30 different proteins and two RNA chains, with total molecular weight of about 1.6×10^6. The largest crystals are of particles from Bacillus stearothermophilus (1.5x0.3x0.2mm), from mutated ribosomal subunits of the same bacteria, in which one protein (L11) is missing (0.8x0.2x0.1mm), and from Halobacterium marismortui (0.6x0.6x0.1mm).

Ribosomes from Halobacterium marismortui are of special interest since they are stable and active at high salt concentrations. Their expected structure should not only contribute to the understanding of the process of protein biosynthesis, but also could be used for comparative structural and functional studies. This may provide insight as to the structural elements which may confer stability in high salt concentrations and for the understanding of the nature of protein-nucleic acids interactions at extremely high salt concentrations, a fundamental problem in molecular-biology for which little data is currently available. Also, since this is an archaebacterium, the comparison of the structure of its ribosome with that of the ribosome from eubacteria may shed light on basic points in evolution.

Because ribosomes from regular sources fall apart at high salt concentrations, their crystals grow from volatile organic solvents which impose many technical difficulties. In contrast, ribosomes from Halobacterium survive and function in high salt concentrations, thus providing a system for obtaining crystals from salts or solid precipitants rather than from alcohols. We were able to grow crystals by vapor diffusion at 19°C from 7% polyethyleneglycol in the presence of 1.2M KCl and 50-150 mM $MgCl_2$. It was observed that the internal order of the crystals is inversely related to the rate of their growth. Best results are obtained when the crystals reach their final size within 2-3 days. Being thin plates, the crystals are rather fragile, and tend to form multi-layer aggregates. However, their mechanical strength can be greatly improved by mild crosslinking and application of a careful seeding technique resulted in obtaining reasonable size single crystals. More sophisticated seeding techniques are currently being employed in order to further improve and increase the crystals.

Using synchrotron radiation, cell dimensions have been determined. Around 0°C the crystals diffract to better than 13Å and last in the X-ray beam for several hours permitting up to 7 exposures from a single crystal. Compact packing is reflected in X-ray patterns as well as in electron micrographs of thin sections of embedded crystals.

PURIFICATION AND CHARACTERIZATION OF RIBOSOMAL PROTEINS FROM THE 30S SUBUNIT OF THE EXTREME HALOPHILE HALOBACTERIUM MARISMORTUI

M. Shoham

Department of Structural Chemistry

The Weizmann Institute of Science Rehovot, Israel

M. Kimura, R. Reinhardt and J. Dijk

Max-Planck-Institut fuer Molekulare Genetik

Abteilung Wittmann

Ihnestr. 63-73

D-1000 Berlin-Dahlem

Ribosomal proteins were extracted from 30S subunits of **Halobacterium marismortui** under native conditions. A high salt concentration (3.2 M KCl) was maintained during all fractionation steps to avoid denaturation. The separation was based on gel filtration and hydrophobic chromatography. It resulted in eight proteins purified to homogeneity and characterized by two-dimensional gel electrophoresis and N-terminal sequences. Three of the fractions contained equimolar protein complexes. The largest complex is one of a 50,000 and a 14,000 dalton protein. Another contained a 1:1 complex between two proteins, tentatively

identified as S14 and S18. In the fraction with the smallest polypeptide there is a dimer composed of two proteins with 11,000 daltons each. The Stokes' radii of the isolated proteins were measured, and the alpha-helical and beta-sheet contents estimated from circular dichroism. Proton NMR data indicate unfolding of the proteins in low salt or in the presence of urea. Protein S5 however, is an exceptionally stable protein, which retains its secondary structure in a salt concentration as low as 0.1 M NaCl, and even in 8M urea.

Several of these proteins have been sequenced. The homology to ribosomal proteins from eubacteria and eucaryotes will be discussed.

X-ray Structural Studies on a 2Fe-2S Ferredoxin from Halobacteria of the Dead Sea

Menachem Shoham, Jerry H. Brown, and Joel L. Sussman

Department of Structural Chemistry
The Weizmann Institute of Science
Rehovot, Israel

There are some bacteria that thrive in the Dead Sea despite its extremely high salt concentration. Moreover, these bacteria have intracellular salt concentrations which exceed even that of the outside environment. In order to help explain the ability of these organisms' proteins to function at extreme salinity, we are studying a 2Fe-2S ferredoxin from Halobacteria of the Dead Sea (HDS) by X-ray crystallographic methods.

This halophilic ferredoxin crystallizes in space group $P6_322$ with unit cell dimensions of a = b = 60.4 Å and c = 127 Å, with one molecule per asymmetric unit. Phase information has been obtained from anomalous scattering (ANO) of the irons and from a Single Isomorphous Replacement (SIR) using a potassium tetracyano platinate derivative. Molecular replacement has also been successfully employed. Real space rotation searches of homologous nonhalophilic ferredoxins - *S. platensis* (Tsukihara et al., J. Biochem. (1981) 90, 1763-1773) & *A. sacrum* (Tsukihara personal communication (1985)) - pivoting around the 2Fe-2S cluster, yielded a single solution.

Chain tracings and electron density map fittings have been carried out, using a real time Vector General computer graphics system connected to a VAX 11/780 computer. Electron density maps based on combined SIR and calculated phases have proven reliable both in locating misplaced groups and substantiating correct features in the chain trace. At present, the molecular model has been refined by CORELS (Sussman et al. Acta Cryst. (1977) A27, 800-804) to an R-factor of 0.28 for the 6 - 2.8 A data.

The bulk of the protein is rather similar to the structure of the nonhalophilic *S. platensis* ferredoxin. Further away from the iron-sulfur cluster, however, the two structures are diverging. The highly acidic N-terminal region, which is absent in nonhalophilic ferredoxins, appears to form a separate, distinct domain, remote from the active site.

SOLUTION STRUCTURE OF HALOPHILIC MALATE DEHYDROGENASE FROM SMALL ANGLE NEUTRON AND X-RAY SCATTERING AND ULTRACENTRIFUGATION MEASUREMENTS

G. Zaccai, Institut Laue Langevin, Grenoble
E. Wachtel and H. Eisenberg, The Weizmann Institute of Science, Rehovot.

The concentration of salt required to maintain the structure and function of enzymes from obligate halophiles would totally inhibit the identical biological function in most other organisms. It is thus of considerable interest to study the structural features enabling this adaptation to extreme conditions.

In this work (1), small angle X-ray and neutron scattering experiments on solutions of malate dehydrogenase from Halobacterium maris mortui (abbrev. hMDH) are analysed together with previously obtained X-ray (2) and hydrodynamic data (3,4) to give a model of the enzyme particle formed by the protein and its interactions with water and salt in the solvent.

The complementarity of information derived from the different experimental techniques is discussed extensively and quantitatively. It derives essentially from contrast variation because it is the difference in density (contrast) between the components of a particle and the solvent that contributes to the information. And since mass density, electron density, and neutron scattering density, are independent of each other, different 'views' of the particle are obtained from the experiments.

In solvents of 1 M to 5 M NaCl hMDH can be approximated by a particle of invariant volume, consisting of a protein dimer (87000 g mol^{-1}) with which are associated 0.85 g of water and 0.35 g of NaCl per gram of protein. The particle has a radius of gyration of 32 Å and an equivalent Stokes radius of 43 Å. The radius of gyration of the protein moiety alone is about 28 Å and that of the salt and water component is about 40 Å. The partial specific volume of the protein calculated, without a priori assumptions, from the data, is constant at 0.753 ml g^{-1} between 1 M and 4 M NaCl, in good agreement with the calculated value. The shape of the protein moiety is similar to the shape of the particle which, at very low resolution, can be approximated by an ellipsoid of axial ratio 1:1:0.6 or 1:1:1.5. At slightly higher resolution, it is clear that both the particle and its protein moiety have an interface with solvent which is significantly larger than that of an ellipsoid. The model proposed has a protein core similar to that of non-halophilic malate dehydrogenase from pig's heart, with about 20% of the protein extending out of the core in interaction with the water and salt component of the particle.

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Halobacterial Glycoproteins

F. Wieland

Institute for Biochemistry, Genetics and Microbiology, University of Regensburg

Regensburg

Halobacteria incorporate sulfate residues into different proteins. The sulfate residues are not reduced, but rather incorporated into glycoconjugates as sulfuric acid esters (1). In vivo studies with $^{35}SO_4^{2-}$ have revealed a low molecular component, the radioactivity of which is incorporated into a set of proteins in a molecular weight range of 26 to 36 KD and into a 200 KD glycoprotein. The 200 KD sulfated protein turned out to be identical with the cell surface glycoprotein of Halobacteria which had been described earlier (2). Structural analysis consistently changed our knowledge of this cell surface glycoprotein. Besides neutral disaccharides (Glc, Gal), it contains two types of sulfated glycoconjugates: i) one high molecular weight sulfated chain made up by a repeating building block. (3,4). This chain resembles the animal glycosaminoglycans, but is synthesized on and transferred to the core protein "en bloc" from a lipid anchor and is linked to the protein via Asn-GalNAc (5). ii) about 10 oligosaccharides containing sulfated glucuronic and iduronic acid, which are linked to the protein via Asn-Glc (6,7). Both N-glycosidic linkage units are different from the hitherto unique N-glycosidic linkage type AsnGlcNAc. Biosynthesis of these glycoconjugates involves sulfated dolichylmonophosphateoligosaccharides from which the completely sulfated carbohydrates are transferred to the core protein (8). This transfer of both types of sulfated carbohydrates to the cell surface glycoprotein occurs at the surface of the halobacterial cell.

The sulfated glycoproteins of intermediate molecular weight (26-36 K) turned out to be constituents of the flagella of Halobacteria. They carry the same type of sulfated oligosaccahrides that was found in the cell surface glycoprotein, and also their linkage unit, Asn-, Glc, turned out to be identical (9). Biosynthesis of these sulfated oligosaccharides showed an unexpected new feature: a peripheral glucose residue of the sulfated dolichol-linked oligosaccharides is transiently methylated during biosynthesis of the glycoprotein. No methylated glucose is found in these glycoconjugates at the protein-linked level (10). A possible function of this transient methylation of an oligosaccharide lipid in the biosynthesis of the glycoproteins will be discussed.

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The effect of lowered Ca++ and NaCl concentration on the cell wall of Halobacterium volcanii from the Dead sea.

**Simone Cohen, *Moshe Shilo, and **Martin Kessel. **Dept. of Membrane and Ultrastructure Research, Hebrew University-Hadassah Medical School, and *Division of Microbial and Molecular Ecology, Inst. of Life Sciences, Hebrew University of Jerusalem.

H. Volcanii isolated from the Dead Sea by Mullakhanbai and Larsen (1975) was classified as belonging to the group of halobacteria despite its moderate NaCl requirement. The bacterium grows optimally at a salt concentration of 2.14M NaCl and 250mM Mg++. Similarly to other known halobacteria the cell envelope of H. volcanii is comprised solely of the cell membrane and a single wall layer of hexagonally arranged cell wall glycoprotein subunits as seen by electron microscopy. The presence of bivalent cations at concentrations of at least 75mM Mg++ or 10mM Ca++ is absolutely required in addition to 2.14M NaCl to maintain the natural pleomorphic shape of the bacteria. Below the above bivalent cation concentrations the cells round up. Envelopes obtained by freezing and thawing of the cells behave similarly and do not show the periodic structure of the cell wall surface after negative staining with 1% uranyl acetate. However, if the envelopes are resuspended in a solution of a bivalent cation only - as low as 1mM Ca++ or between 10 and 40mM Mg++ - the envelopes do not round up and the surface pattern is visible.

Envelopes in 2.14M NaCl and 250mM Mg++ "dissolve" when resuspended in water and leave only small membrane fragments which do not show a periodic pattern. However, envelopes first resuspended in 10mM Ca++ solution only, survive a resuspension in water. If the envelopes are resuspended first in 1mM Ca++ only small fragments with patches of periodic structure remain. Further resuspension in water causes dissolution of the envelope.

The addition of 2.14M NaCl to the envelopes resuspended in less than 20mM Ca++ causes the envelopes to round up, generally seal, and to lose the periodic structure. When the Ca++ concentration is less than 1mM the addition of NaCl does not result in the formation of sealed membranes.

Apparently only a very low concentration of bivalent cations is necessary to maintain the periodic structure on the cell envelope of this bacteria. This feature is common to the the S-layer of many eubacteria. The presence of NaCl at a concentration needed for growth counteracts the protective effect of the bivalent cations and increases the minimal concentration required to maintain the initial shape of the envelope and the integrity of the cell wall.

Analysis of the high resolution structure of the cell envelopes under the conditions described above was performed using the SPIDER Image Processing System as designed by Joachim Frank at the Wadsworth Center for Laboratories and Research, the New York State Dept. of Health, Albany, N.Y.

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Dielectric Properties of some Halophilic Bacteria

by Ben-Zion Ginzburg and Hywel Morgan

Plant Biophysical Laboratory

The Hebrew University of Jerusalem

Dielectric properties of suspensions of the halophilic bacteria H. marismortui and H. halobium over a frequency range of 100 kHz to 250 MHz are presented. Based on the assumption that the membrane is of a low conductivity at low frequencies typical Maxwell-Wagner dielectric dispersions are observed. Values for the conductivity and permittivity of the cell interior and cell membrane are presented for both species.

Water sorption isotherms at several temperatures are also presented for ferridoxin - an enzyme isolated from the H. marismortui cytoplasm, together with H and S of the sorbed water as a function of hydration.

Structure and Function of the Retinal Pigments in Halobacteria

Walther Stoeckenius
Department of Biochemistry and Biophysics
and
Cardiovascular Research Institute
University of California, San Francisco

In their natural habitat halobacteria are exposed not only to high salt concentrations but also to high light intensities and low O_2 concentrations. They have adapted to this environment in part by using retinal pigments, which resemble the visual pigments of animals, and function as light sensors and light energy transducers. The main energy source of the extreme halophiles is respiration, which generates an electrochemical proton gradient across the cell membrane. This gradient in turn drives ATP-synthesis, uptake of K^+, expulsion of Na^+, Ca^{2+}, and locomotion. The cytoplasma K^+ concentration roughly equals outside Na^+ concentration, and since Na^+ and K^+ are present in molar concentrations, large amounts of energy can be stored in these ion gradients. They can maintain a membrane potential for a long time in the absence of external energy sources, and they perform vital functions; the Na^+ gradient for instance drives uptake of amino acids, the main substrate of respiration. Light also generates an electrochemical proton gradient mediated by the 25KD retinylidene protein, bacteriorhodopsin (bR), which is present in the cell membrane and functions as a light-driven proton pump, using 520 to 620 nm light effectively. A second very similar retinylidene protein, halorhodopsin (hR), also functions as a light-driven ion pump and mediates the uptake of Cl^-, the main interior anion; its presumably respiration-driven counterpart has not been identified.

The light energy transducers bR and hR are complemented by additional retinal pigments which are photosensors and allow the cells to find an environment

where the long wavelength light used by the energy transducers dominates the spectrum. The best investigated of the signal transducers is sensory rhodopsin (sR), which mediates a repellent response to near UV light and an attractant response to green and red light. A repellent response in the blue region can be attributed to a separate retinal pigment. These sensory pigments closely resemble the energy transducers in most of their physical and chemical properties, but are not electrogenic ion pumps; how they transmit signals to the flagellar motor is not known.

In H. halobium hR is present in lower concentrations than bR and the sensory pigments in still lower concentration, amounting to about $5x10^3$ molecules/cell for sR, $2x10^4$ for hR and $2x10^5$ for bR for cells when grown under conditions to maximize the production of bR. In most pigmented halobacteria isolated from natural environments, the dominant retinal pigment is also bR, but few wild strains contain it in the high amounts and in the form of crystalline membrane patches extensively studied in the laboratory strains of H. halobium, H. cutirubrum and H. salinarium. In a survey of nearly 50 haloalkaliphilic strains no bR-like pigment was detected, but in most strains we found pigments closely resembling hR and the blue light receptor of H. halobium and have characterized them spectroscopically.

The extensive and diverse use of retinal pigments by extreme halophiles cannot be explained by their unusual environment, because bR and hR can function at low salt concentrations and very similar sensory retinal pigments are widespread in the animal kingdom. A speculative answer to this problem may be found in the early evolution of life.

THE MECHANISM OF ACTIVE CHLORIDE ACCUMULATION IN HALOBACTERIUM HALOBIUM. Janos K. Lanyi, Department of Physiology and Biophysics, University of California, Irvine, CA 92717, U.S.A.

Since osmotic balance in halobacteria is maintained by intracellular KCl against an external medium which contains mostly NaCl, growth of these organisms (and therefore enlargement of cytoplasmic space) depends on the accumulation of potassium and chloride ions from the medium. It seems likely that potassium is accumulated passively, driven by the inside negative electrical potential across the cytoplasmic membrane, but chloride must be taken up by an active mechanism, i.e. one which will cause chloride flux against an unfavorable electrochemical potential. Halorhodopsin, a retinal protein found in the cytoplasmic membrane of halobacteria, constitutes such an active chloride transport system. Chloride transport will be driven by this system in right-side-out cell envelope vesicles suspended in sulfate plus chloride, and the anion uptake then generates both the expected electrical potential and inside vs. outside chloride concentration difference (Schobert and Lanyi, 1982).

Halorhodopsin is a purple-blue pigment of relatively low molecular weight (25 kD), a retinal protein similar in this respect to bacteriorhodopsin. Halorhodopsin will bind various anions at two distinct sites, with small spectroscopic shifts as a consequence. The more specific of these sites binds only chloride and bromide (and iodide), and upon binding the absorption band shifts from 565 to 578 nm. Important clues as to the molecular details of this reversible chloride-binding, and the chloride-translocation which follows it after absorption of a photon, are furnished by the observable photochemical reactions of the pigment, and their dependency on chloride. These photoreactions constitute the "photocycle" of halorhodopsin, which has an overall turn-over time of about 15 msec. During this time, two relatively long-lived intermediates are produced from the parent HR(578).Cl: HR(520).Cl and HR(640). HR(565) produces only HR(640) upon illumination. These processes have been analyzed a by steady-state model (Oesterhelt et al, 1985) and a single turn-over model (Lanyi et al, 1985), and the results agree with a proposed scheme (Oesterhelt et al, 1985) in which HR(520).Cl decays into HR(640) by loss of a chloride, and HR(640) decays back to HR(565) independently of chloride concentration. HR(565) is then reconverted into HR(578).Cl through binding of chloride. Estimation of the rate constants of several of these reactions was possible, using the single turn-over model and flash-spectroscopy. From the results it can be calculated that while the binding of chloride by HR(565) is with high affinity, the binding of chloride by HR(640) is with very much reduced affinity. This is most likely a consequence of the fact that the isomeric configuration of retinal in HR(565) is all-

trans, but in HR(640) it is 13-cis, as demonstrated by a variety of methods, including flash spectroscopy in the near UV (Lanyi, 1984; Lanyi et al, 1985). In vesicles it is known that HR(565) binds chloride from the outside medium. Present experimentation is aimed to discover whether HR(640) binds chloride from the interior space. If the latter is found, the proposed photocycle and its chloride dependency contains all the requirements for an active chloride transport mechanism.

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TRYPTOPHAN IMAGING OF BACTERIO-OPSIN RECONSTITUTED INTO LIPID VESICLES.

*A.M. Kleinfeld, +J.D. LeGrange and +S.R. Caplan, *Harvard Medical School, Boston, MA 02115 and +Weizmann Institute of Science, Rehovot, Israel.

The spatial distribution of tryptophanyl residues of reconstituted bacterio-opsin (bO) was determined by resonance energy transfer (RET) from Trp to the n-(9-anthroyloxy) (AO) fatty acids ($2 \leq n \leq 16$). Purple membrane was bleached, retinal was extracted and the solubilized protein was reconstituted into liposomes of egg phosphatidylcholine by dialysis. Reaction of the reconstituted bO with n-bromosuccinimide (NBS) was used to assess the Trp quantum yield heterogeneity. The results indicate that 3 to 4 of the 8 Trp are only weakly fluorescent. Steady state anisotropy measurements were carried out on vesicles treated with amounts of NBS sufficient to quench 0, 25, and 75% of the Trp fluorescence and results from each sample are consistent with significant Trp mobility ($r(300\ nm) < 0.20$). Energy transfer was measured for each of eight different AO probes, added to the vesicles at fatty acid to lipid ratios between 0.5 and 5%. RET efficiencies (T) were determined from the quenching of Trp fluorescence intensity as well as the sensitized emission of the AO probes. T values were found to increase almost linearly with probe position and the increase with concentration was in excellent agreement with that expected for RET. In all cases the average T values obtained from Trp quenching were about twice those from sensitized emission, as expected from the observed Trp quantum yield heterogeneity. A Monte Carlo analysis of the T values (Kleinfeld, 1985, Biochemistry 24, 1874) indicates that the fluorescent Trp are distributed deep within the bilayer.

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The Metal Binding Site of Bacteriorhdopsin (bR)

M. Engelhard, K.-D. Kohl and B. Hess
Max-Planck-Institut für Ernährungsphysiologie, Dortmund

Bacteriorhodopsin (BR) the purple pigment of Halobacterium Halobium converts light energy into a proton concentration gradient. The absorption maximum at 568 nm can be shifted to 605 nm by removing specifically bound cations. At least one divalent cation attached to the protein is a necessary prerequisite for the function of bR. Mößbauer spectroscopy of a sample with incorporated $^{57}Fe^{2+}$ reveals a low spin binding site. This binding site was covalently modified with organic cations and with $(Co(NH_3)_5OH_2)^{3+}$. Subsequent analysis of the labelled peptides enabled us to determine the sequence of the binding site. EXAFS-studies of a similar preparation revealed a rigid iron attached to the protein with 6 to 8 coordinated oxygens.

D.J. Kushner, Department of Biology, University of Ottawa, Ottawa, Canada, K1N 6N5. "Studies of active transport as a means of understanding salt requirements and salt tolerance".

Cells make contact with their external environment through the cytoplasmic membrane. In bacteria, this membrane serves as the site of active transport and the closely-linked process of energy production. Na^+ ions are required for active transport in marine, moderately halophilic and extremely halophilic bacteria. The action of Na^+ seems to be mediated through Na^+/H^+ antiport, and Na^+/substrate symport in these organisms (1 - 3).

Transport may also be used to study the basis of salt tolerance, which is generally less well understood than salt requirement. Higher concentrations of NaCl inhibit α-amino isobutyric acid (AIB) transport by V. costicola. Cells grown in 1.0 m or higher NaCl can transport AIB in the presence of higher NaCl concentrations than those grown in 0.5 M NaCl. Cells grown in 0.5 M NaCl and then exposed to 1.0 M NaCl can develop a more salt-tolerant active transport of AIB without growth or protein synthesis.

Two levels of salt-tolerant AIB transport were observed: to 3 M and to 4 M NaCl. Tolerance to 4 M NaCl required both C and N sources, was accompanied by increased intracellular solutes, and was lost on exposure of cells to 0.5 M NaCl. In contrast, tolerance to 3 M NaCl could develop without C and N sources, was not accompanied by increased intracellular solutes and was not lost when cells were suspended in 0.5 M NaCl (4).

Kinetic studies have shown that high NaCl concentrations do not act on an AIB-specific transport system but, rather, on cellular energetics, probably on the maintenance of an adequate proton motive force. The effects of adding substances such as betaine, proline and glutamate on the development of salt-tolerant transport will be described. It is known that growing V. costicola in higher NaCl concentrations leads to increased intracellular betaine contents (5), changes in membrane lipids (6) and in a specific outer membrane protein (7). The relation of such changes to increased salt-tolerant transport will be discussed.

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^{31}P NMR Studies of Dunaliella

Michal Bental*, Ami Ben Amotz♯, Mordhay Avron♯ and Hadassa Degani*
Isotope (*) and Biochemistry (♯) Departments
The Weizmann Institute of Science
76100 Rehovot, Israel

With the development, in recent years, of high resolution in vivo NMR spectroscopy, biological research had acquired a powerful tool to probe noninvasively the internal cellular metabolites and their environment. In particular, ^{31}P-NMR monitors the cellular phosphate compounds that reflect the energy profile of the living cell.

We employ in vivo NMR spectroscopy in the study of Dunaliella Salina. The unicellular photosynthetic algae of the genus Dunaliella are unique in their ability to grow in a wide range (0.1 - 5.0 M) of salt concentrations. The main osmoregulatory processes of glycerol synthesis and elimination were previously studied by ^{13}C- and ^{1}H-NMR of intact cells (Degani et al., 1985, BBA 846, 313-323). In this report, we describe ^{31}P-NMR studies of the intracellular phosphates of D. Salina and their variation with salt concentration and the concentration of PO_4^{-3} in the growth medium.

During the measurements the cells were kept in the dark under aerobic conditions. At 4°C their phosphate spectrum remains constant over at least 6 hours. When the cells are cultured under optimal growth conditions (1 M NaCl, 0.2 mM PO_4^{-3}), the following prominent resonances appear in the ^{31}P-NMR spectrum (Fig. 1A): (1) phosphomonoesters (PME), including glycerol phosphate and other sugar phosphates; (2) inorganic phosphate in the growth medium (Pi_{ext}); (3) intracellular inorganic phosphate (Pi_{int}); (4) an unidentified peak (x); two large signals of tripolyphosphate (PPP) - one due to the two terminal phosphate groups and the other, at a higher field, due to the middle phosphate; (6) ATP signals due to α- and β-ATP. The γ-ATP is masked by the large PPP signal; (7) a signal due to medium (4-10 atoms) polyphosphate chains (Poly P). The spectrum of cells kept under unaerobic conditions at 37°C for several hours becomes dominated by very intense, broad peaks of Pi and polyphosphate chains. The appearance of these signals under stress conditions reflects the presence of high concentrations of NMR invisible phosphate in the living cells. This phosphate is probably stored as insoluble, long chain polyphosphaste deposits.

Studies of cells grown at three different PO_4^{-3} concentrations (0.05, 0.2 and 1 mM) showed that in excess PO_4^{-3} (1 mM) the spectrum remains similar to that obtained from cells grown at optimal (0.2 mM) PO_4^{-3} concentration (Fig. 1A). However, in cells cultured under phosphate limited conditions (0.05 mM) a large increase in the intracellular Pi and a complete disappearance of the tripolyphosphate signals were observed.

Comparison of ^{31}P spectra of cells grown at four different NaCl concentrations (0.5, 1.0, 2.0 and 4.0 M) showed that the composition and amount of phosphate metabolites in cells grown at 0.5 M and 1.0 M NaCl is similar (Fig. 1A). However, under high salt concentration - 2 M or 4 M NaCl, a change occurs in the amount of tripolyphosphate which decreases to a non-detectable level (Fig. 1B). This absence of tripolyphosphate occurs also when the high salt medium contains excess PO_4^{-3} (1 mM).

A major finding of this study is the detection of the presence of tripolyphosphates in D. Salina under optimal growth conditions. Furthermore, the results suggest that phosphate metabolism, and in particular that of the tripolyphosphates, is regulated by both the osmotic conditions and phosphate availability. These regulatory processes in the cells are currently under investigation.

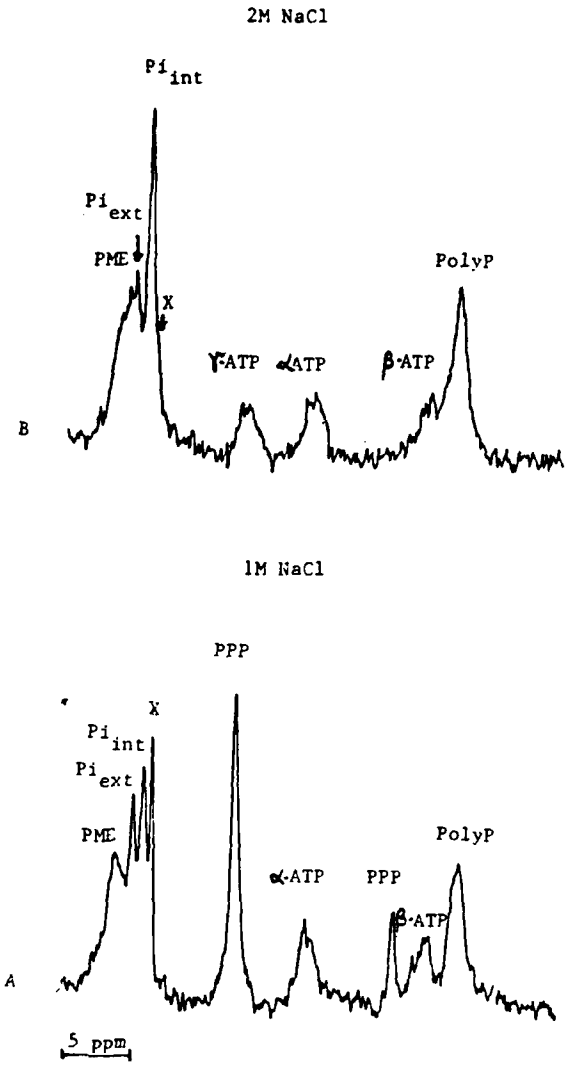

Fig. 1. 121.5 MHz ^{31}P-NMR spectra of Dunaliella Salina under aerobic conditions at 4°C. Algae were grown in medium containing 0.2 mM PO_4^{-3}, 1 M NaCl (A) or 0.2 mM PO_4^{-3} and 2 M NaCl (B).

Halobacterial motion and taxis

D. Oesterhelt, M. Alam and W. Marwan

Max-Planck-Institut für Biochemie, D-8033 Martinsried

Halobacteria and square bacteria have right-handed helical flagella which consist of bundles of several filaments (1,2). Clockwise and counterclockwise rotations can be observed by dark field microscopy and cause forward and backward swimming of the cells. When the sense of rotation changes the flagellar bundle does not fly apart as in E.coli. Cell cultures, especially those of mutant strain M-175 produce during the stationary phase the so-called super flagella which are aggregates of loose flagella and are usually 10 to 20 times longer than the cell body. The constituting flagellins have apparent molecular weights of about 23,000, 26,000 and 31,000 Da and are glycoproteins carrying the same sulfated oligosaccharide moieties as the cell surface glycoprotein of halobacteria (3). They can be purified by in vitro dissociation and reconstitution of flagellar filaments which, depending on the conditions of pH, temperature and ionic strength, show polymorphic transition between normal, curly, straight and ring forms.

Analysis of spontaneous reversals of the swimming cell reveals a difference to the behaviour of E.coli. It leads to a kinetic model of the switching process in the halobacterial flagellar motor. The signal transduction chain in

the cell regulating the switch in the motor was studied with the help of two blue light receptors, sensory rhodopsin and system P_{470}. Flash and double flash experiments demonstrate that the minimal response time, even under light saturation, is about 1 sec and that signal formation occurs in the dark involving one catalytic step. By production of giant cells and micro beam stimuli it could be shown that the signal is not transmitted via a membrane bound process and that the radius of light action is limited to about 3 um around the flagella. In addition, no coordination of different flagella is observed in the photophobic response of giant cells excluding a long range action of the signal itself.

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PHOTOTAXIS IN HALOBACTERIUM HALOBIUM. E.K. Wolff, R.A. Bogomolni, B. Hess, W. Stoeckenius: Max-Planck Institute, Dortmund, W. Germany and Dept. of Biochemistry and Biophysics, University of California, San Francisco, CA 94143.

H. halobium is attracted by long wavelength and repelled by short wavelength light. Sensory rhodopsin (sR) has been identified as a receptor for both responses. Absorption of light in its 587nm absorption band leads to the attractant response and also generates a photointermediate absorbing at 373nm (S_{373}). When this is photoconverted back to sR_{587}, a repellent response results. We have used a light microscope to record the responses. A suspension of cells is illuminated through a dark-field condensor with >750nm light, which is not perceived by the cells. Shorter wavelength background light can be added. Through the objective lens, a 150µm spot of light, which can be varied in wavelength and intensity, is projected into the center of the field and the >750nm light scattered from the cells in this spot is monitored by a photomultiplier (PM). The PM current is proportional to the number of cells in the spot and its initial rate of change when the light intensity and/or wavelength in the measuring spot are changed, measures the reactivity of the cells. The resulting action spectra show a broad peak between 560nm and 620nm with one or two subsidiary maxima for the attractant response and an even broader peak in the blue and near UV for the repellent response. The spectral width of this repellent response and the point of crossover from attractant to repellent response depends on wavelength and intensity of background light and the cell strain used as well as growth conditions. Without background light, the crossover usually occurs between 460 and 480nm and for a mutant lacking the major carotenoids, at 535nm but even higher crossover points can be obtained under different growth conditions. Such cells show a negligible long wavelength attractant response and a strong repellent response to blue light, which does not require a long wavelength background. These observations are incompatible with sR_{587} and S_{373} as the only photoreceptors and suggests a second repellent receptor absorbing near 480nm, which we have also detected spectroscopically. Its photoreaction cycle has a halftime slightly shorter than that of sR and its longest-living photoproduct has an absorption maximum at shorter wavelength than S_{373}.

ABSTRACT

NaCl Stimulated Physiological Changs in Salt Tolerant Bacteria

Russell H. Vreeland

Recently developed lines of evidence indicate that salt tolerant (euryhaline) bacteria have the ability to physiologically adapt to different NaCl concentrations. Electron microscopic studies have shown that the cell walls of Halomonas elongata are relatively loose and unstable following low salt growth but in high salts the walls are extremely tight and stable. Analyses of the phospholipid content of H. elongata and fatty acid compositions of twelve different euryhaline organisms show that the organisms responses to NaCl are remarkably similar. H. elongata and several other bacteria appear to contain more negatively charged phospholipids following high salt growth. In addition analyses of the fatty acid composition of these bacteria show that the organisms produce significantly more unsaturated and cyclopropanoic fatty acids in high salts than they do in low salts. Differences between the protein profiles of low and high salt grown cells have also been demonstrated. These physiological changes as well as others currently under study indicate that the mechanism of salt tolerance in euryhaline bacteria is very complex and involves more than just establishing simple osmotic equilibrium with compatible solutes. The evidence collected to date on these organisms indicates that much of their adaptation involves extensive cell wall changes. These changes provide an excellent model for studies on cell wall dynamics and the function of the wall in relation to the salt tolerance of an organism.

The Role of Monovalent Cations in the Regulation of Respiration of a Halotolerant Microorganism

Shifra Ken Dror and Y. Avi-Dor
Department of Biology, TECHNION-Israel Institute of Technology
Haifa 32000, Israel

In the halotolerant bacterium, Ba_1, monovalent cations display a profound effect on the rate of respiration. K^+ and Na^+ ions stimulated oxygen uptake in the acid and in the alkaline pH-range respectively. Simultaneous presence of both cations maintained a high and constant respiratory rate throughout the whole pH-range investigated (pH 6.5-8.5). K^+ and Na^+ was found to be involved in Ba_1, like in some other microorganisms (1, 2, 3), in the generation of cross-membranal proton cycles. The latter may contribute to the regulation of the intracellular pH. Indeed, the K^+-dependent rise in oxygen uptake at low pH was shown to be accompanied by intracellular alkalinisation when the Na^+-linked substrate, ethanol, was the electron donor. Uncouplers or inhibitors of energy transfer suppressed the effect of K^+ on respiration, as well as on the intracellular pH. Thus, apparently, some step connected with the above effects required ATP. K^+ in Ba_1, has no direct effect on the respiratory chain since the oxidation of NADH by inverted vesicles derived from cells of Ba_1 was not accelerated. The stimulatory effect of Na_1 on ethanol

oxidation, in contrast to that of K^+, was insensitive to uncouplers and to inhibitors of energy transfer. Moreover, Na^+ also stimulated NADH oxidation catalysed by inverted membrane vesicles. Thus, Na^+ must act directly on some component in the respiratory chain.

Spectrophotometric and electron spin resonance techniques revealed that Na^+ stimulated the ubiquinone reduction/oxidation step by facilitating the transfer of the second electron to the quinone. Na^+-dependent stimulation of the NADH-quinone oxidase, due to acceleration of the conversion of the quinone radical to hydroquinone has formerly been reported to occur in the marine microorganism, Vibrio alginolyticus (4, 5). Ba_1 (6), like the Vibrio (7) is able to extrude Na^+ against an electrochemical gradient with the aid of a respiration-linked, uncoupler insensitive primary Na^+ pump. The site of the stimulation of the respiration probably corresponds in both microorganisms to the site of the Na^+ translocation. In summary, we suggest that control of the intracellular pH is the major factor in the regulatory function of K^+-ions on the respiratory activity of Ba_1. In the case of Na^+, in addition to the control of the intracellular pH, a direct stimulatory effect on electron transport must be taken into consideration, i.e. release of the kinetic control in the NADH-quinone oxido-reductase segment of the respiratory chain.

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M. Kogut & N.J. Russell Department of Biochemistry, University College Cardiff, S. Wales, U.K.

Title: Phenotypic adaptation of a moderate halophile to altered salinity and osmotic stress - the role of phospholipids

Our studies are concerned with the way in which halophilic bacteria sense and respond to changes in salinity. We have reasoned that the sensing/effector systems might exist in the cell envelope of Gram-negative bacteria (Russell & Kogut, 1985). It was known already that moderate halophiles and halotolerant bacteria contain a greater proportion of negatively charged phospholipids in their membranes when they are grown at higher salt concentrations; we showed that this was true of the moderate halophile Vibrio costicola in which the major change is a relative increase in phosphatidylglycerol compared with phosphatidylethanolamine (Hanna et al., 1984).

We found that following a sudden increase (shift-up) in salt concentration from 1M to 3M, there is a lag in growth and protein synthesis followed by gradual adoption of the new growth rate appropriateto the new salt concentration. During this lag period the ratio of neutral to negatively charged phospholipids also changes to that characteristic for the higher salt concentration (Kogut & Russell, 1984). This change in phospholipid composition is brought about by alterations in the rates of phospholipid synthesis rather than degradation (Russell et al., 1985).

Because the phospholipid changes appeared to be an integral part of haloadaptation we have studied this phenomenon as a means of determining more about the sensing/effector system. In a series of shift-up experiments in which the size of the shift (2- or 3-fold increase in NaCl concentration) and the final concentration were varied, we found that it is the magnitude of change in external solute concentration that governs the adaptive lag and the extent of change in phospholipid synthesis, whilst the final salt concentration also has some influence, especially on the growth rate. This suggested to us that there might be an osmotic component operating. We have confirmed this by carrying out shift-up experiments in which medium osmolarity was raised with sucrose or glycerol. In the experiments where osmolarity was increased by addition of sucrose the phospholipid changes were very similar to those where osmolarity was increased by NaCl. However, this was not the case when the osmolarity was raised by addition of glycerol. The cultures adapted much more readily to growth in glycerol and the pattern of phospholipid synthesis was different: the rate of phosphatidylethanolamine synthesis remained higher than that of phosphatidylglycerol throughout. It is not possible to substitute all salt with uncharged solute and we are investigating the ionic dependence of the adaptive response.

Phospholipid biosynthetic enzymes are membrane bound, and we are investigating their regulation by salt and non-ionic solutes, to determine which enzymes are involved in haloadaptation.

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Abstract: Mechanism of Halotolerance in Dunaliella: Determination of glycerol and inorganic ion content. Ami BEN-AMOTZ, Department of Biochemistry, The Weizmann Institute of Science, Rehovot, Israel.

The unicellular flagellate Dunaliella has the remarkable capacity to grow in media ranging in NaCl concentration from about 0.1M up to 5M. The major means of osmoregulation of Dunaliella is by production of intracellular glycerol at concentrations which are proportional to the extracellular NaCl concentration. However, due to the considerable disagreement as to the contribution of inorganic ions to the internal osmotic pressure, we used new methods to determine the cellular volume and the ionic content of Dunaliella. The results showed that the intracellular ionic concentration of algae cultured at 1-4M NaCl is 20-100mM Na^+, 150-300mM K^+, 20-50mM Mg^{++}, 1-3mM Ca^{++} and 500-1000mM PO_4^{--}.

The results suggest that Dunaliella accumulates K^+ and PO_4^{--} and eliminates Na^+ by cellular metabolic control. The contribution of total inorganic ions to the internal osmotic pressure of Dunaliella is low the cells maintain isoosmotic intracellular concentration by producing and accumulating glycerol.

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Evidence for the metabolic control of Na^+ and Cl^- in Dunaliella grown at constant salinity.

Margaret Ginzburg and Ben-Zion Ginzburg

Plant Biophysical Laboratory, The Hebrew University of Jerusalem.

The green unicellular alga Dunaliella is to be found in most saline and hypersaline waters in the temperate zones of the world. Cells of this genus are characterized by the ability to adapt rapidly to changes in salinity. Among the adaptations enabling the cells to withstand salt are a high intracellular glycerol content and powerful ion pumps controlling the cell concentrations of Na^+ and Cl^-. These latter are the subject of this paper.

The concentrations of Na^+ and Cl^- within Dunaliella cells are approximately one-third of the concentrations in the surrounding medium (Ginzburg and Ginzburg 1985a and b). There is increasing evidence to show that cell Na^+ and Cl^- are not distributed evenly throughout the cell. Instead, $\geq$ 30% of the cell volume contains the ions at concentrations close to those outside and can easily be washed free of salt, while the bulk of the cell volume (~70% of the total) contains Na^+ and Cl^- at concentrations of around 135 mM (outside concentration 1500 mM) when the cells are illuminated (Ginzburg and Richman, 1985). In the dark the inside and outside concentrations become equalised (Ginzburg and Ginzburg, to be published). A qualitatively similar picture of cells in the light has been obtained by X-ray microprobe (Hajibagheri and Gilmour, to be published). When cells are illuminated after a period in the dark, they lose Na^+ and Cl^-, provided that the medium contain inorganic phosphate and K^+.

The above experiments have been performed on Dunaliella species belonging to the two major forms of the genus, D. parva 19/9 (small green cells) and D. salina (large cells with high carotene content). Experiments with D. salina were performed in media containing 3 M NaCl; on illumination, the overall Na^+ and Cl^- concentrations fell by nearly 2 M in the space of 2 hours.

Loss of glycerol at night has been demonstrated by Avron & Ben-Amotz (1978).

The results point to a diurnal shift in solute content of Dunaliella cells: gain of Na^+ and Cl^- with loss of glycerol at night; the reverse effects during the day. Glycerol is a product of photosynthesis. The energy needed to bring about loss of Na^+ and Cl^- is very likely derived from the breakdown of ATP formed during photosynthesis.

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Genome and Gene Structures in Halobacteria

K. Ebert, W. Goebel, C. Hanke, A. Moritz, U. Rdest and B. Surek
Institut für Genetik und Mikrobiologie, Universität Würzburg,
8700 Würzburg, W.-Germany

The genome of Halobacterium halobium can be separated into two fractions according to the G+C content. Fraction I (FI) which represents 70 - 90 % of the total DNA contains 68 % G+C while fraction II (FII) is A+T richer (58 % G+C) and comprises 11 - 30 % of the total DNA. Extrachromosomally, one plasmid (pHH1) (160 kb in size) is present in several copies containing 58 % G+C, thus being part of FII. It carries most known halobacterial insertion elements (ISH) which are at least in part responsible for the high genetic variability of H. halobium. A collection of heterogeneous cccDNA molecules which may derive from chromosomal sequences, mostly from FII (Minor Circular DNA, MCD) are found in H. halobium and other halobacteria.

DNAs from a number of new rod-shaped, purple-membrane forming isolates share high homology and show an identical G+C content of 68 % in FI, but their FII DNA varies from strain to strain in G+C content and quantity. These strains lack pHH1 and differ in their plasmids. Those strains which do not carry pHH1 sequences are genetically more stable than those harbouring pHH1 or pHH1-related plasmids.

Despite the considerable genetic divergence in rod-shaped and square bacteria, there are also DNA sequences in common. Among those we characterized more closely the insertion element ISH26 and the gene for 7S RNA.

ISH26 exists in two modifications. One is 1500 bp in size and flanked by 16 bp inverted repeats. The second is enlarged by 206 bp following the 16 bp sequence. A stretch of 78 bp of the core region is directly repeated in the additional 206 bp sequence and another 16 bp sequence flanks this composite IS element.

All halobacteria synthesize an abundant non-ribosomal stable RNA of about 7S. In all rod-shaped halobacteria tested this RNA is 304 nucleotides in length. In square and pleomorphic bacteria, 7S RNA is enlarged by 30 nucleotides. In H. halobium the gene encoding 7S RNA is colinear with the 7S RNA and shows in the 3'flanking region two G+C rich stem and loop structures and sequence homology in the corresponding region of the 5S RNA gene. The 7S RNA has a largely double stranded structure reminiscent of the mammalian 7SL RNA.

Some halobacteria harbour a small plasmid of 1.7 kb, present in appr. 1000 copies per cell. The mini plasmid contains single restriction sites for HindIII and SalI and may thus be useful vectors for gene cloning in halobacteria. A shuttle vector for E. coli and halobacteria was constructed which consists of pBR322, the CAT gene of E. coli and in front of CAT the promoter region of the 7S RNA gene. This construct is presently tested in transformation experiments.

TRANSFER RNA GENE STRUCTURE AND TRANSCRIPT PROCESSING IN THE ARCHAEBACTERIUM *Halobacterium volcanii*

C.J. DANIELS

Dept. Microbiology, The Ohio State University, Columbus, Ohio 43210, USA

ABSTRACT

In studies aimed at elucidating the structure and controls for gene expression in the archaebacteria we have cloned and sequenced six tRNA genes from the halophilic strain *Halobacterium volcanii*; $tRNA^{Cys}$, $tRNA^{Lys}$, $tRNA^{Ser}$, $tRNA^{Val}$, initiator $tRNA^{Met}$ and $tRNA^{Trp}$ (1,2). One of these genes, $tRNA^{Trp}$, was found to have a 105 bp intron located at the second base 3' to the anticodon. Evidence that this sequence represents a functional gene in this organism consists of the observations that (i) the predicted exon sequences are identical to that of the $tRNA^{Trp}$ RNA sequence; (ii) both the exon and intron sequences are present once in the genome and (iii) these cells contain detectable amounts of a 180 nucleotide, intron-containing precursor transcript and a 105 nucleotide excised intron fragment. We have also observed that both exon and intron sequences are highly conserved among all members of the halobacteria examined. Initial characterization of the RNA processing mechanism in the halophiles was examined *in vitro* using a substrate generated from the phage T7 polymerase transcription system. Extracts from *H. volcanii* produced two major products with this substrate; one at 105 nucleotides corresponding in size to the excised intron and a more stable 76 nucleotide species corresponding in size to the mature tRNA. In contrast to the yeast tRNA processing system, this reaction did not require ATP and was not capable of incorporating $^{32}PO_4$ from γ [32p] ATP into the tRNA-sized product. Comparisons of the 5' flanking sequences of these genes has revealed the presence of four highly conserved sequence blocks which may function as transcriptional control signals. The possibility that those sequences have a role in the expression of small RNAs is strengthened by the observation that these four blocks, in the same relative positions, are also present in the 5' flanking region of the *H. halobium* 7S RNA gene (3).

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Genetic Transfer in *Halobacterium volcanii*

Moshe Mevarech, Ilan Rozenshein, Ruth Werczberger, Tal Zusman
Department of Microbiology, George S. Wise Faculty of Life Sciences,
Tel-Aviv University, Tel-Aviv.

In an attempt to develop a genetic system for the extreme halophilic bacterium *H. volcanii* auxotrophic mutants as well as mutants resistant to various antibiotics were isolated. The auxotrophic mutants were induced, while some of the antibiotic resistant mutants occur spontaneously at a frequency of 10^{-9} .

We were able to show that mutants grown as a mixed culture on a solid support transfer genetic markers. The frequency of recovery of prototrophic recombinants is 10^{-6} . The transfer is insensitive to DNase and requires the presence of the two parental strains in a viable state, thus, the transfer resembles conjugation more than transformation. Polarity in this genetic transfer had not been established, since no distinction could be observed between donors and recipients.

Currently, we are adapting various approaches towards the understanding of the mechanism of the transfer as well as the applicability of the transfer as a mean for genetic mapping. Multiply marked strains are being constructed in an effort to study the kinetics and the polarity of the transfer. In addition, the stability of the recombinants and their state of ploidy is studied.

ABSTRACT

Transfer RNAs of Halobacterium volcanii
Ramesh Gupta
Department of Medical Biochemistry, and
Department of Chemistry and Biochemistry
Southern Illinois University
Carbondale, IL 62901, U.S.A.

The extreme halophilic bacteria belong to the kingdom - Archaebacteria, which is distinct from the other two kingdoms - Eubacteria and Eukaryotes. The transfer RNAs (tRNAs) of several eubacteria and eukaryotes have been sequenced, which show that certain features of the tRNAs are specific to one or the other kingdom. To determine whether archaebacterial tRNAs are similar to the eubacterial or to the eukaryotic tRNAs, or are unique, a set of tRNAs from an extreme halophilic bacterium have been characterized. A total of 41 tRNAs of Halobacterium volcanii have been identified and sequenced by a combination of methods. This set includes at least one tRNA for each of the 20 amino acids. These are five tRNAs for Leu, four for Gly, three each for Ala, Arg, Pro, and Ser, two each for Glu, Ile, Lys, Met (initiator and non-initiator), Thr, and Val, and one each for the remaining eight amino acids. In principle, these tRNAs can cover at least 55 codons out of the possible 61 codons for the 20 amino acids. This set of archaebacterial tRNA sequences can be compared with nearly complete sets of tRNA sequences of eubacteria and eukaryotes (Escherichia coli, yeasts, and mammals). The H. volcanii tRNAs lack the modified residues - ribothymidine, 7-methylguanosine and dihydrouridine, which are present in nearly all of the eubacterial and the eukaryotic tRNAs. A unique modification, 1-methylpseudouridine is present in H. volcanii tRNAs. It replaces ribothymidine of the other tRNAs. H. volcanii tRNAs, though follow general tRNA patterns in terms of both the sequences and the modification patterns, they are similar to tRNAs of eubacteria in some respects, to those of eukaryotes in others, while in yet other ways they are quite distinct. As in the eukaryotes only Leu

and Ser tRNAs are class II (having large extra arm). The initiator tRNA is unique in having a 5'-triphosphorylated end, and has certain features which are similar to those of the other archaebacterial initiators, but are distinct from the initiators of the eubacteria and the eukaryotes. Some of the special features of H. volcanii tRNAs may be archaebacterial characteristics, which others may be due to the very high internal salt concentration of the extreme halophiles.

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ORGANIZATION OF THE HALOBACTERIUM HALOBIUM GENOME

Felicitas Pfeifer
Max-Planck-Institut für Biochemie, 8033 Martinsried,
Federal Republic of Germany

The genome of Halobacterium halobium NRC817 can be fractionated into two DNA components of different guanosine plus cytosine (G+C) content. The major DNA fraction (FI) comprises 70% of total DNA and contains the more G+C rich chromosomal sequences (68 mol%). DNA sequences of significantly lower G+C content (58 mol%) constitute fraction FII. Heterogeneous collections of cccDNA as well as some chromosomal DNA sequences are found in this latter fraction.

FII DNA of H. halobium exhibits an unusually high structural variability. Multiple copies of various insertion elements (ISH1, ISH2, ISH23, ISH24, ISH26, ISH27 and ISH28) are found predominantly in this DNA fraction. The three FII DNA components pHH1, minor cccDNA and chromosomal FII DNA islands have been investigated in more detail.

(1) A 160 kb DNA fragment of the H. halobium chromosome containing 70 kb of FII DNA could be analysed by a "chromosome walk" using the FII DNA copy of the insertion element ISH1 as a starting point. Copies of several insertion elements (ISH1, ISH2, ISH26 and ISH27) as well as at least 10 other repeated sequences are clustered in the 70 kb "island" of FII DNA. The flanking FI DNA sequences contain a larger portion of unique sequences. The 70 kb FII DNA island is not present in the genome of purple membrane producing independent isolates GN101, GRA, YC81819-9 and SB3.

(2) pHH1, the main 150 kb species of cccDNA in H. halobium NRC817 is present in 6-8 copies per genome. The high structural variability of pHH1 in various phenotypic mutants is due to insertions, deletions and rearrangements. The four independent isolates mentioned above contain no sequences homologous to pHH1. cccDNAs of these strains remain unaltered in phenotypic mutants.

(3) In addition to pHH1, cccDNA of H. halobium NRC817 contains species of very low copy numbers ("minor cccDNA"). Size determinations indicate that in wild type these cccDNA species range in size from 60 kb to 180 kb. Some phenotypic mutants contain minor cccDNA species of smaller

Sizes (10 kb - 30 kb). Several of these sequences could be cloned and characterized in more detail. S83, GN101 and YC81819-9 contain minor cccDNAs homologous to H. halobium sequences.

The occurrence of H. halobium minor cccDNA sequences in all independent isolates contrasts with the absence of pHH1 and the 70 kb FII DNA island. Thus, the latter two FII DNA components can be regarded as characteristic genomic component of H. halobium whereas sequences present in minor cccDNA are more conserved among purple membrane producing halobacteria.

Only a few copies of H. halobium insertion elements are present in these independent isolates. The lack of high frequency genomic rearrangements in these strains indicates that the structural dynamics of the H. halobium genome is not a characteristic feature of halobacteria but confined to certain strains.

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Molecular Characterisation of a Multi-Copy Plasmid Isolated from a Halophilic Bacteria LPR3 SP.

R. Sanchez-Lopez, I. Hoffman-Kuhn and B. Jarry,
Laboratoire de Génétique Moléculaire des Eucaryotes
CNRS, 67085 Strasbourg, France

LPR3 SP is a gram-negative bacteria which can grow in a wide range of salt concentrations (5 to 25 % NaCl). In order to study, at genetic level, the mechanisms of salt adaptation we have analyzed the membrane and soluble proteins synthesized in various growth conditions. We have also isolated a small 4,1 Kb long multi-copy plasmid. Molecular characterization of the plasmid has been performed by cloning in E. Coli. Surprisingly several regions of the plasmid DNA can be efficiently transcribed and translated in the heterologous host. Evidence will be presented that at least one of these genes is also active in SPR3 SP. Sequence analysis of the promotor region will be described and compared with E. Coli transcription signals. This analysis opens the way to the preparation of a shuttle vector replicating in E. Coli and LPR3 SP and therefore to the genetic analysis of salt adaptation of this halophilic bacteria.

GENETIC REGULATION OF BACTERIO-OPSIN SYNTHESIS IN HALOBACTERIUM HALOBIUM

Mary Betlach, Diane Leong, and Herbert Boyer. Department of Biochemistry and Biophysics, University of California, San Francisco, California 94143-0554.

We are investigating regulation of the gene encoding bacterio-opsin (bop) which is the protein moiety of bacteriorhodopsin, a light activated proton pump found in the purple membrane of H. halobium. On limitation of the oxygen concentration, bacteriorhodopsin levels in the cell increase up to five fold (Sumper et al., 1976. FEBS Lett. 69, 149-152). The bacterio-opsin gene has been cloned and characterized (Dunn et al., 1981, Proc. Nat. Acad. Sci. 78, 6744-6748; DasSarma et al., 1984, Proc. Nat. Acad Sci. 81, 125-129). A second gene (brp) affecting bacterio-opsin synthesis is the site of insertions resulting in a Bop$^-$ phenotype. This gene is located 526 bp upstream of the bop gene and is transcribed in the opposite direction (Betlach, et al., 1984, Nulceic Acids Res. 12, 7949-7959). Significant homology between 120 bp regions upstream of the transcription initiation sites of bop and brp genes could be indicative of shared promoter and/or regulatory signals. Comparison of bop and brp transcripts in brp gene inactivated Bop$^-$ mutants suggest some degree of regulation of the bop gene at the transcriptional level. The possibility of transcriptional regulation is also supported by current studies in which there are significant increases in mRNA levels in wild type cells grown under conditions of low oxygen tension. A secondary structural prediction of the putative brp protein reveals 6-7 hydrophobic α helices of sufficient length to span the membrane (Betlach et al., 1984). Synthetic peptides extrapolated from the brp gene DNA sequence have been used as antigens for the generation of antisera. These antisera are being used to identify and isolate the putative brp protein in order to determine its role in bop gene expression.

Gene expression of Halobacterium halobium phage ØH

Felix Gropp and Wolfram Zillig

Max-Planck-Institut für Biochemie, 8033 Martinsried bei München, Federal Republic of Germany

In eubacteria, phages have served as instructive models for studying gene expression. Among archaebacterial viruses the temperate bacteriophage ØH of Halobacterium halobium is one of the most extensively studied on the molecular level. Its 59 kb double-stranded DNA genome is terminally redundant and partially circularly permuted due to a headful packaging mechanism. The prophage genome is a covalently closed circle of 57 kb. A circularized part of the phage genome, the L plasmid, confers immunity against ØH infection to its host. A spontaneous ØH mutant, ØHL1, which carries the insertion element ISH50 in its L region, is able to overcome the L plasmid immunity but not the immunity imparted by the whole prophage. Thus, immunity is conferred on at least two different levels, one encoded within the L region, the other outside (Schnabel et al. 1982a, Schnabel et al. 1982b, Schnabel 1984, Schnabel and Zillig 1984).

We have investigated the transcription of the genome of this phage. Like other bacteriophage genomes, that of phage ØH is expressed following a specific time schedule. Under standard conditions the latency period is about 7h. Most prominently a 3.7 kb piece close to one end of the L region, that piece of the phage genome that coincides with the early transcripts, and the insertion element ISH1.8 which is also expressed in uninfected cells, are transcribed. The switch from early to late transcription is blocked by puromycin, indicating that a newly synthesized protein is required.

A segment close to the other end of the L region is not expressed in the lytic cycle. In the immune H. halobium strain R1(L) carrying the L plasmid as sole part of the ØH genome, this region is strongly transcribed, whereas the lytic 3.7 kb transcript is not detectable. It thus appears possible that the 3.7 kb transcript might be involved in the switch for early to late gene expression and the two transcripts from the other side of the

L region are involved in conferring immunity. Insertion of ISH50 must in some way circumvent this immunity block. In the true lysogen R1(ØH1) two additional regions outside L are transcribed and could be involved in establishing the second level of immunity not overcome by ØHL1.

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Molecular Characterization of Genes Encoding Elements of the Halobacterium Protein Synthesis Apparatus

Patrick P. Dennis
The University of British Columbia, Vancouver, B.C., Canada

The protein synthesis apparatus of archaebacteria exhibits many unique features which distinguish this group of organisms from eukaryotes and eubacteria. In an attempt to understand the molecular nature of some of these unique features, we have begun to clone and characterize genes encoding components of the protein synthesis apparatus. The chromosome of H. cutirubrum contains a single ribosomal RNA gene cluster. The 5' to 3' organization of genes within this six kilobase pair region is: 16S, alanine tRNA, 23S, 5S, cysteine tRNA. The entire gene cluster is transcribed as a single long primary transcript; processing of mature RNA sequences from the 5' region of the transcript begins prior to the completion of synthesis at the 3' end. There are five conserved octanucleotide direct repeats (TGCGAACG) in the 900 base pair 5' flanking sequence in front of the 16S gene. The positions of these repeat sequences correspond to the different 5' ends of the primary transcript and probably represent the RNA polymerase start sites. The 16S and 23S rRNA genes are surrounded by long nearly perfect inverted repeat sequences. These sequences probably form duplex structures in the primary transcript and are recognized by an RNaseIII-like endonuclease activity that carries out the initial excision of the precursor 16S and 23S rRNA sequences. These precursors are rapidly trimmed to the mature 16S and 23S molecules and assembled into ribosomal particles. The processing sites for 5S rRNA appears to be at or very near to the mature ends of the 5S molecule. The tRNA sequences are processed with reduced efficiency from the primary transcript. Nuclease cuts have been detected at the ends as well as in the middle of the cysteine tRNA sequence suggesting that there may be alternative processing pathways, one resulting in proper excision of the mature tRNA sequence and the other resulting in improper excision and degradation of the tRNA sequence. The transcription termination sequence is believed to be at or beyond an AT-rich sequence preceded by a GC-rich sequence located distal to the cysteine tRNA gene. Two genes encoding ribosomal protein HcL20 and HcL11 have been cloned on a 60 Kb fragment of genomic DNA. Characterization of these genes, their organization and their in vivo transcripts is in progress.

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LIST OF PARTICIPANTS

AVI-DOR Y.
Department of Biology
Technion-Israel Institute of Technology
Haifa 32 000
Israel

BEN AMOTZ A.
Department of Biochemistry
Weizmann Institute of Science
Rehovot 76 100
Israel

BETLACH M.
Department of Biochemistry and Biophysics
School of Medicine
University of California San Francisco
San Francisco CA 94143,
U.S.A.

CAPLAN S.R.
Department of Membrane Research
Weizmann Institute of Science
Rehovot 76 100
Israel

DANIELS C. J.
Department of Microbiology
Ohio State University
Columbus OH 43210
U.S.A.

DEGANI H.
Department of Isotope Research
Weizmann Institute of Science
Rehovot 76 100
Israel

DENNIS P.P.
Department of Biochemistry
The University of British Columbia
Vancouver, B.C.
Canada V6T 1W5

EISENBERG H.
Polymer Department
Weizmann Institute of Science
Rehovot 76 100
Israel

ENGELHARD M.	Max Planck Institut für Ernährungsphysiologie D-4600 Dortmund FRG
GINZBURG B. Z.	Plant Biophysical laboratory Hebrew University, Giv'at Ram Jerusalem 91 904 Israel from February 1986 Department of Biochemistry Oxford University Oxford OX1 3QU England
GRANT W.D.	Department of Microbiology Medical Science Building University of Leicester Leicester LE1 7RH England
GROPP F.	Max Planck Institut für Biochemie Abt. Zillig D-8033 Martinsried bei München FRG
GUPTA R.	Medical Biochemistry Neckers Building School of Medicine Southern Illinois University at Carbondale Carbondale, Illinois 62901 U.S.A.
HAYGOOD M.	ONR 800 N. Quincy St. Arlington, VA 22217-5000 U.S.A.
HOCHSTEIN L. I.	239-10 Ames Research Center Moffett Field CA 94035 U.S.A.
JAVOR B.	Scripps Institute of Oceanography A-002 University of California San Diego La Jolla, CA 92093 U.S.A.

KESSEL M.
Dept. of Membrane and Ultrastructure Research
Hebrew University
Hadassah Medical School
Jerusalem 91 010
Israel

KOGUT M.
Department of Biochemistry
Queen Elizabeth College
London W8 7AH
England

KUSHNER D.J.
Department of Biology
University of Ottawa, Ottawa
Canada K1N 6N5

LANYI J. K.
Department of Physiology and Biophysics
California College of Medicine
University of California Irvine
Irvine CA 92717
U.S.A.

MATHESON A.T.
Department of Biochemistry and Microbiology
University of Victoria
Victoria, British Columbia
V8W 2Y2
Canada

MEVARECH M.
Department of Microbiology
Faculty of Life Sciences
Tel-Aviv University
Tel-Aviv 69 978
On sabbatical
Department of Microbiology
M330 Biological Sciences Building
University of Alberta, Edmonton
Canada T6G 2E9

MORGAN H.
Plant Biophysical Laboratory
The Hebrew University, Giv'at Ram
Jerusalem 91 904
Israel

OESTERHELT D.
Max Planck Institut für Biochemie
D-8033 Martinsried bei München
FRG

OREN A.	Division of Microbial and Molecular Ecology Institute of Life Sciences Hebrew University Jerusalem 91904 Israel
OTTOLENGHI M.	Vice President for Research and Development Hebrew University Jerusalem Israel
PARKS L.	Department of Microbiology North Carolina State University P.O.Box 7615 Raleigh, NC 27695-7615 U.S.A.
PFEIFER F.	Max Planck Institut für Biochemie Abt. Zillig D-8033 Martinsried bei München FRG
RDEST U.	Institut für Genetik und Mikrobiologie der Universitat Würzburg 87 Würzburg FRG
ROTEM Z.	United States-Israel Binational Science Foundation 2 Alharizi St. Jerusalem 91 076 Israel
RUSSELL N.J.	Department of Biochemistry University College P.O.Box 78 Cardiff CF1 1XL U.K.
SAALFELD, F.E.	Director of Research Programs, ONR 800 N. Quincy St. Arlington, VA 22217-5000 U.S.A.

SANCHEZ-LOPEZ R.
Laboratoire de Genetique
Moleculaire des Eukaryotes
11, Rue Humann
67085 Strasbourg Cedex
France

SHILO M.
Division of Microbial and Molecular Ecology
Institute of Life Sciences
Hebrew University
Jerusalem 91904
Israel

SHOHAM M.
Department of Structural Chemistry
Weizmann Institute of Science
Rehovot 76 100
Israel

SIMON R. D.
Department of Biology
State University College of Arts and Science
Geneseo, N.Y. 14454
U.S.A.

STOECKENIUS W.
Cardiovascular Research Institute
University of California
San Francisco, CA 94143
U.S.A.

SUSSMAN J. L.
Department of Structural Chemistry
Weizmann Institute of Science
Rehovot 76 100
Israel

VENEZKI D.L.
Scientific Director, ONR
223 Old Marylebone Road
London NW1 5TH
England

VREELAND R. H.
Department of Biological Sciences
University of New Orleans
Lakefront New Orleans, Louisiana 70148
U.S.A.

WERBER M.
Medical Laboratories
Meir Hospital
Kfar Saba 44281
Israel

WIELAND F.	Department of Biochemistry Stanford University School of Medicine Stanford CA 94305 U.S.A.
WITTMANN H.G.	Max Planck Institut für Molekulare Genetik D-1000 Berlin 33 (Dahlem) F.R.G.
WOLFF E.K.	Max Planck Institut für Ernährungsphysiologie D-4600 Dortmund, FRG
YONATH A.	Department of Structural Chemistry Weizmann Institute of Science Rehovot 76 100 Israel
ZACCAI G.	Institut Laue Langevin 156X-38042 Grenoble Cedex France

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